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14. ABSTRACT

Xavier University of Louisiana is in the unique position of developing capability in drug discovery especially in the areas of cancer and health disparities. A significant proportion of the funded research on Xavier's campus including collaborative projects involving Tulane University are related to cancer, drug design, synthesis, and drug delivery. This project expands the partnership between Xavier University and Tulane Cancer Center to develop and validate drugs for breast cancer therapy. The Drug Design Team at Xavier consists of experts in computer aided drug design methods and synthesis and has formed a productive partnership with the Cancer Drug Validation Team at the Tulane Cancer Center. This inter-university collaboration involves training Xavier researchers, including undergraduate students, to carryout the experiments necessary to determine if new compounds would be suitable as new drugs to treat breast cancer. Three separate studies are ongoing as subprojects: (1) Design and synthesis of novel ceramide analogs that potentially reverse chemotherapy drug resistance, (2) Design and synthesis of small molecule inhibitors of HER2 tyrosine kinase to suppress tumorigenesis, and (3) Identification of compounds with the potential for estrogen receptor activity.

15. SUBJECT TERMS

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INTRODUCTION:

This project brings together the skills and resources of Xavier University and Tulane University researchers to form a collaborative team in the area of drug design and validation. This inter-university collaboration whereby a number of *in vitro* and *in vivo* approaches will be applied to validation of lead compounds designed and synthesized at Xavier will involve training of Xavier researchers and students in drug target validation, biological assays of drug efficacy, evaluation of resistance pathways, and identification of synergistic drug combinations. Xavier researchers, Drs. Thomas Wiese and Jayalakshmi Sridhar are experts in computer aided drug design methods, and Dr. Maryam Foroozesh is an expert in the design and synthesis of small biologically active molecules. Tulane Cancer Center researchers, Dr. Frank Jones is an expert in HER2 positive breast cancers and has an active drug validation program, Dr. Barbara Beckman and Dr. Matthew Burow, are experts in the area of endocrine resistant breast cancer. The distinct contributions of each institute will place this collaboration in a unique position to successfully design and validate drugs to address the most pressing challenges in breast cancer therapy. The specific aims of this collaborative project are to develop, promote, and sustain independent, competitive breast cancer research at Xavier while developing a true partnership between the two institutions.

BODY:

Foroozesh/Beckman/Burow Subproject (Novel Ceramide Analogs as Anti-Cancer Agents)

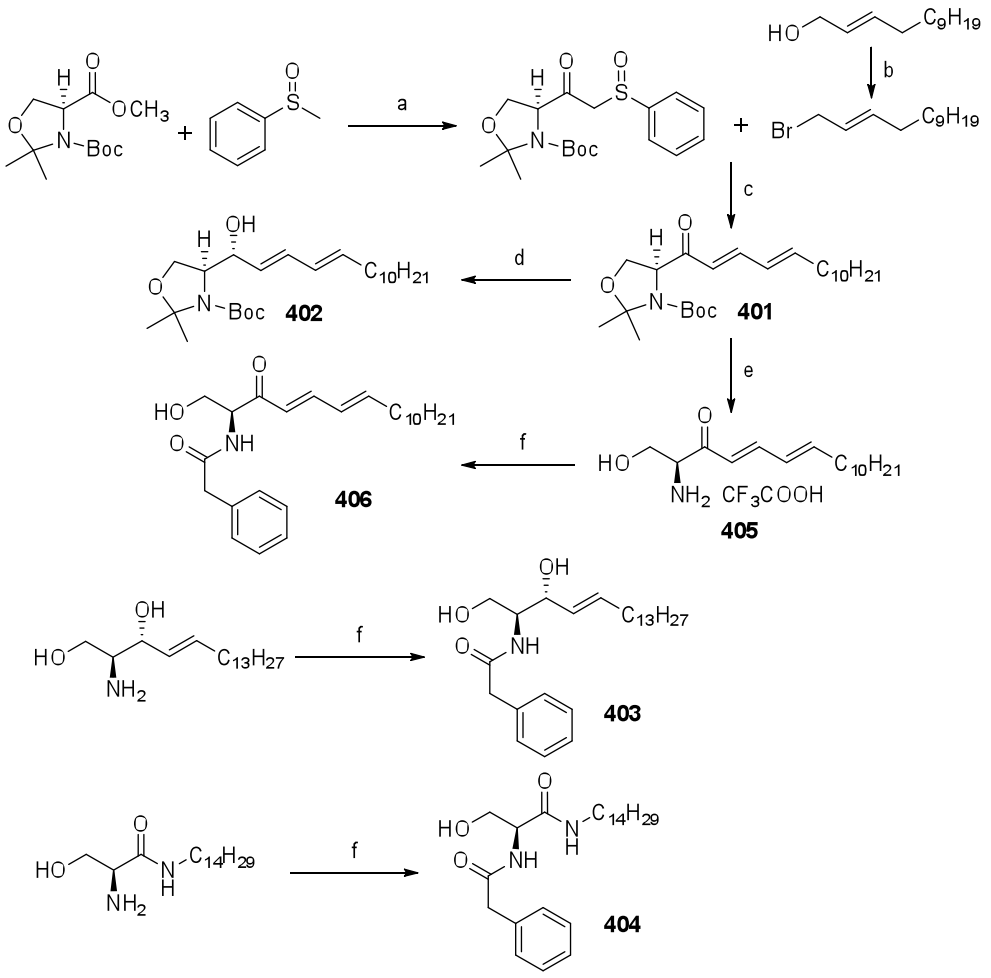
The research accomplishments of this subproject include the following syntheses and bioassays:

Design and Synthesis of Ceramide Analogs

Task 1- Hire research associate to assist in project (Month 1)

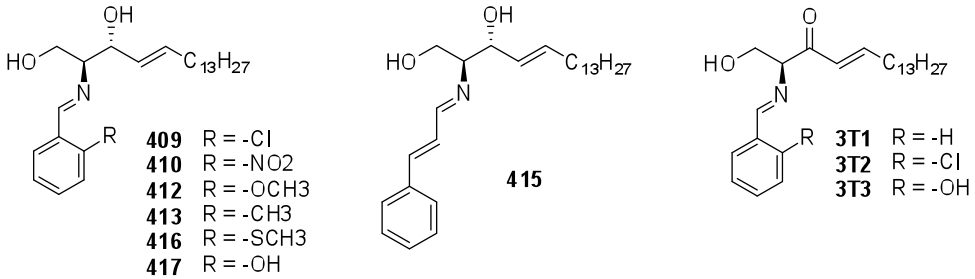
Dr. Jiawang Liu who has worked in Dr. Foroozesh's research lab since 2009 was assigned to work on the DoD project. He is an expert in the design, synthesis, and bioassays of biologically active molecules.

Task 2- The synthesis of ceramide analogs (401, 402, 403, 404 and 406) with different backbones were achieved based on the previously published methods.[1, 2] These specific analogs were designed to help us identify the contributions of the ceramide backbone in the anti-cancer activities. The synthetic scheme for this group of analogs is shown bellow.



Synthesis scheme for analogs 401-406

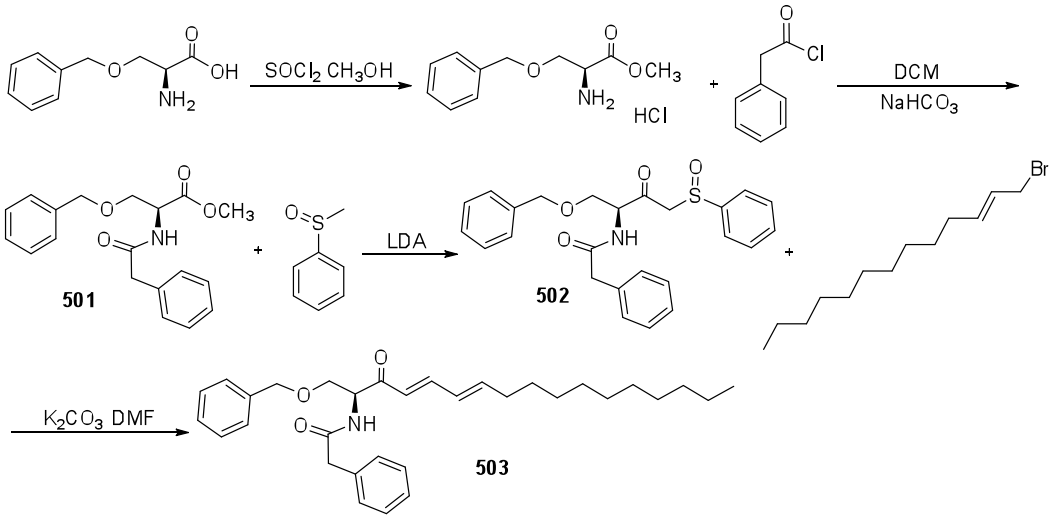
Task 3- Ten ceramide analogs containing the imine group (409, 410, 412, 413, 415, 416, 417, 3T1, 3T2, and 3T3) were prepared through a facile reaction.[3] Since one of our previously synthesized ceramide analogs containing an imine group has shown the most potent anti-cancer activities,[1] the modifications here are expected to increase the anti-cancer activity and/or provide us with important structure-activity relationship information. The structures of these compounds are shown bellow.



Structures of ceramide analogs 409, 410, 412, 413, 415, 416, 417, 3T1, 3T2, and 3T3

Task 4- A ceramide analog with the1-hydoxy group blocked (503) was prepared using relatively inexpensive staring materials and simple synthetic route as shown bellow.

Compared with the previous synthetic methods,[2] our new synthetic route avoids the usage of expensive starting material methyl (S)-(-)-3-Boc-2,2-dimethyl-4-oxazolidinecarboxylate and excessive protecting and de-protecting steps. Using the starting material O-benzyl-serine, the 1-position blocked ceramide analog (503) was synthesized through a facile four-step route shown bellow.



The four-step synthetic route used for the synthesis of analog 503

Determination of Anti-Cancer Activities of the Ceramide Analogs

Task 1- The anti-cancer activities of ceramide analogs 401, 402, 403, 404, and 406 were tested using a cellular viability assay and a clonogenic survival assay in MCF-7, MDA-MB-231, and MCF-7TN-R cells. Compounds 401 and 406 were the most effective compounds across all cell lines with an IC_{50} value of 4.05 ± 1.3 μM ($p<0.001$) and 4.26 ± 1.48 μM ($p<0.001$) respectively, in the chemo-resistant MCF-7TN-R cell line. Interestingly, IC_{50} values for all analogs except analog 401 were lower in the chemo-resistant MCF-7TN-R and hormone therapy-resistant MDA-MB-231 cell lines, indicating that these compounds exhibit increased therapeutic potential in drug-resistant cancers (Table 1). The fact that two compounds with the 3-ketone-4,6-diene backbone (406 and 401) have shown the most potent anti-cancer activities in this group suggests that the 3-hydroxy-4-ene backbone is not necessary for bioactivity of ceramides as previously believed.[4] The raw results (Figures 1 and 2) are shown bellow.

Our results in apoptosis assays show that analog 406 induces a 4.3 ± 1.1 fold ($p<0.05$) increase over control in the induction of apoptosis, compared to C8-Cer with a 2.34 ± 0.79 fold increase. Analog 406-induced cell death is mediated through the intrinsic apoptotic pathways, with 3.59 ± 0.45 ($p<0.05$) fold increase in caspase-9 activity following treatment with the analog. In conjunction with our previous studies, these results suggest that development of ceramide analogs with a diene component in the sphingosine backbone may be well suited for the treatment of chemo-resistant breast cancer.

Table 1. IC_{50} values of ceramide analogs in the MTT viability assay and the clonogenic survival assay (μM). The values are the means of three independent experiments.

	IC_{50} values in viability assay (μM)			IC_{50} values in survival assay (μM)		
	MCF-7	MCF-7-NTR	MDA-MB-231	MCF-7	MCF-7-NTR	MDA-MB-231
401	3.906	4.047	26.76	5.07	1.854	1.454
402	26.44	9.908	45.54	5.692	5.185	3.174
403	37.28	4.742	35.79	4.175	5.62	1.585
404	233.6	28.96	NE	10.16	10.05	17.55
406	22.03	4.263	81.94	3.403	1.808	1.402

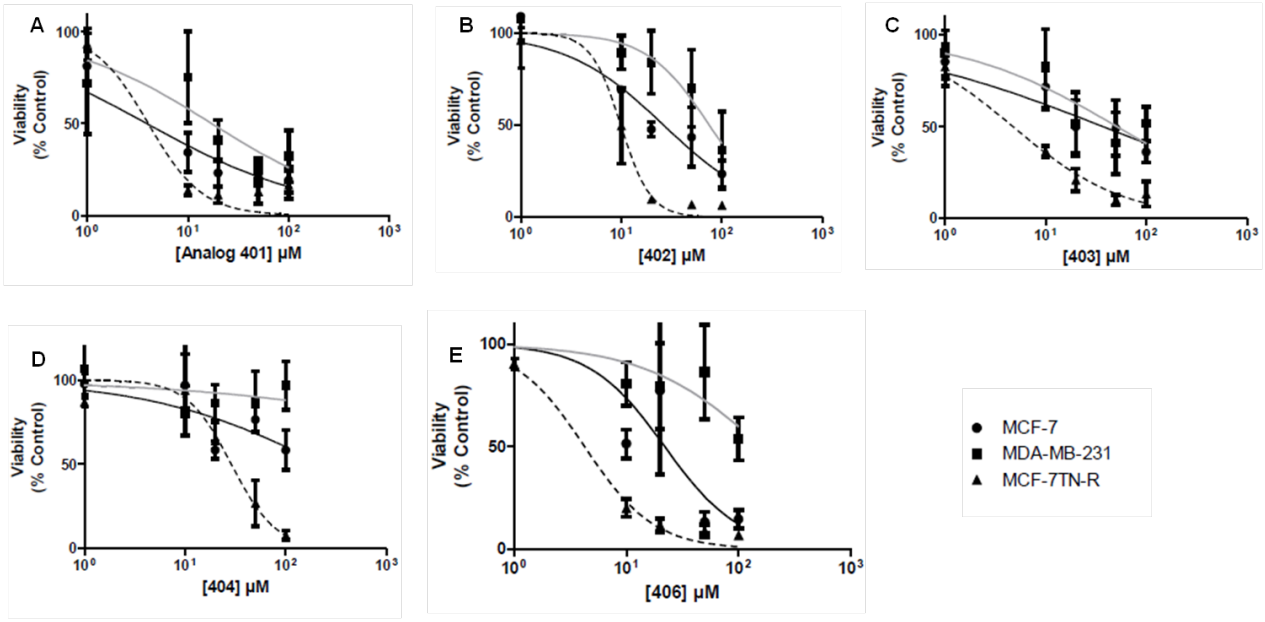


Figure 1. Effect of ceramide analogs on breast cancer viability. MCF-7, MDA-MB-231, and MCF-7TN-R cells were treated with increasing concentrations of analogs for 24h. The values are the mean ±SE of three independent experiments.

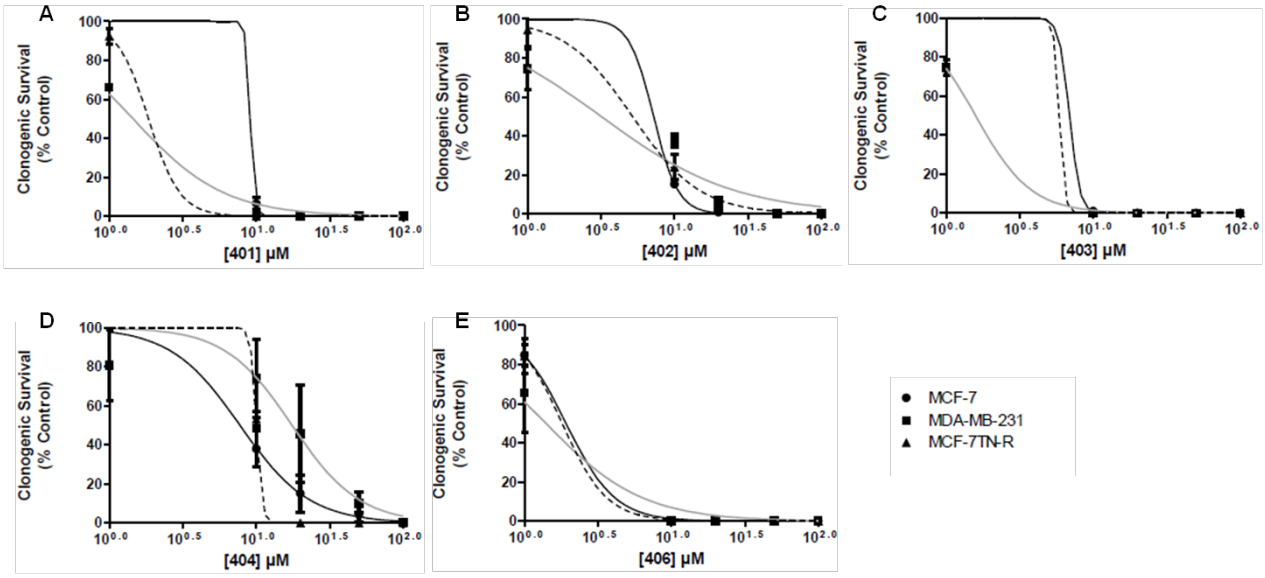


Figure 2. Effect of ceramide analogs on breast cancer clonogenic survival. MCF-7**,** MDA-MB-231, and MCF-7TN-R cells were treated with increasing concentrations of analogs and allowed to grow until colony formation was noted (generally 10-12 days). The values are the mean ± SE of three independent experiments.

Task 2- A longitudinal activity comparison of analogs 315, 406, 415 and 503 was performed. These cell viability assays were performed on NCI/ADR-RES, NCI/ADR, OVCAR8, MCF-7 and MCF-7/Dox cells. The results showed that among these analogs, compounds 406 and 415 show the most potent activities. The raw data is provided bellow in Figure 3.

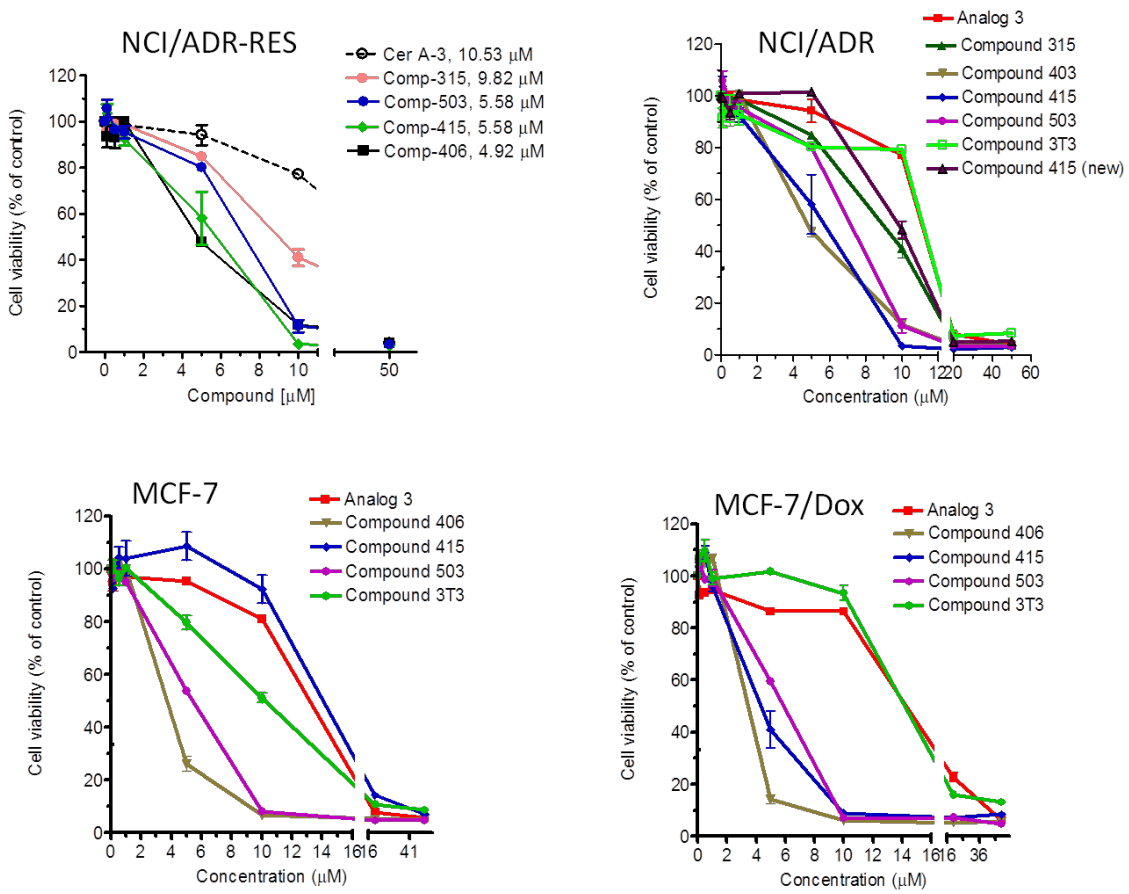


Figure 3. Effect of ceramide analogs on cancer viability. NCI/ADR-RES, NCI/ADR, MCF-7, and MCF-7/Dox cells were treated with increasing concentrations of analogs for 72 h. Analyzed by CellTiter-Glo luminescent cell viability assay from the Promega.

Task 3- Glucosylceramide synthase (GCS) inhibition assays showed that analog 406 has a mild or no GCS inhibition activity in OVCAR8, NCI/ADR-RES and NCI/ADR cells. This observation suggests that cytotoxic activity of analog 406 is not a result of the inhibition of GCS enzyme. On the other hand, analog 503 showed a significant GCS inhibition activity in all of the tested cell lines. This observation confirms our hypothesis that GCS activity can be inhibited through modification of ceramide's 1-postion. These results provide us with a great perspective for designing novel GCS inhibitors. GCS is considered to be a critical enzyme implicated in cancer drug-resistance. The results of the GCS activity assays are shown bellow in Figure 4.

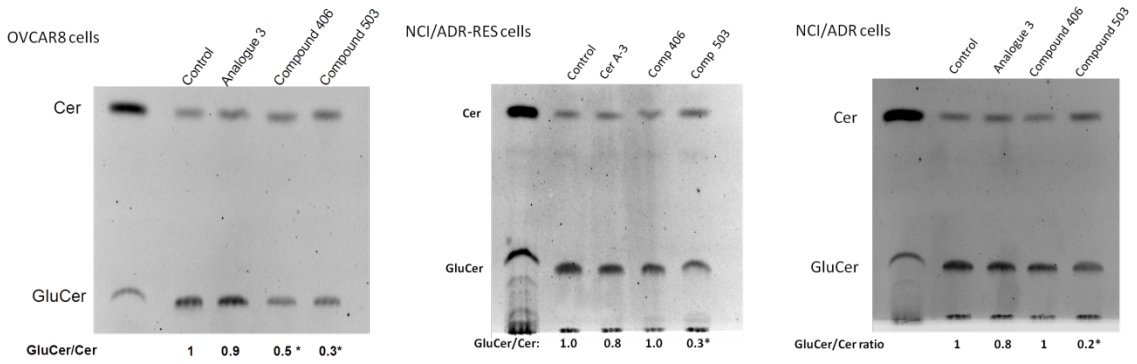


Figure 4. GCS inhibition activity assay in OVCAR8, NCI/ADR-RES, and NCI/ADR cells. The figures showed the thin layer chromatograph (TLC) results of each test. Treatments for 48 hours in 5% FBS RPMI-1640 medium; analyzed by fluorescence enzymatic assay (Gupta V et al *J Lipid Res* 2010, 51:866-74), three times.

We are currently working on one manuscript entitled "Design, Synthesis, and Characterization of 4,6-Diene-ceramide Analogues for the Treatment of Chemo-resistant Breast Cancer" co-authored by Adharsh Ponnapakam, Jiawang Liu, Barbara Drew, Tony Wang, James Antoon, Thong Nguyen, Patrick Dupart, Maryam Foroozesh, and Barbara Beckman (to be submitted as a Brief article to Journal of Medicinal Chemistry in September 2012). James Antoon received his M.D. in May 2012 after receiving his Ph.D. in 2010 at Tulane University. He is doing his residency in pediatrics at the University of North Carolina. Barbara Drew is in her residency in obstetrics and gynecology in Connecticut. Tony Wang is a second year medical student at Tulane University working on this DoD subproject along with Adharsh Ponnapakam who is now a Masters student in pharmacology at Tulane University. They will continue the work in Dr. Beckman's laboratory. Dr. Jiawang Liu (a postdoctoral fellow) and Patrick Dupart (a technician, Xavier Alumni) work in Dr. Foroozesh's lab at Xavier University.

Wiese/Burow Subproject (Identification of novel estrogens and antiestrogens in the USDA Phytochemical and FDA Marketed Drugs databases)

Research accomplishments of this subproject in Y1 include the following tasks in Specific Aim 1:

Develop structure-based pharmacophore models and ligand-receptor (docking) models for estrogens based on the crystal structures of ER alpha and beta (with bound agonists or antagonists) and then virtually screen the USDA Phytochemical, Chinese Herbal Medicine, and the FDA Marketed Drug Databases for new estrogens.

Task 1- Identify student to assist in project (Month 1)

Pharmacy student Chioma Obih who had worked in the Wiese lab for the previous 2 years was assigned to this project in Fall 2011. She is supported by the College of Pharmacy Center of Excellence Grant focused on providing significant research training

for pharmacy students. Dr. Wiese has trained her in structure based modeling methods using the MOE software and she worked with Dr. Wiese on Task 2.

Task 2- Develop structure-based pharmacophore models for estrogens (Months 1-4)

2a - Obtain all crystal structures of ER LBDs (month 1)

A search of the Protein Database in fall 2011 resulted in the identification of 62 crystal structures of the human Estrogen Receptor (ER) ligand-binding domain (LBD) all of which contained one bound ligand. These LBD structures were processed and aligned relative to each other so that similarities and differences in ligand-binding pockets could be identified.

2b - Sort LBD structures by cavity shape and helix-12 position (month 1-3).

In preliminary studies prior to this project, we have shown that ligand receptor docking (or virtual screening using docking) can produce very different results between ER LBD structures containing steroid or stilbene ligands, even though both ligands are agonists and the LBD cavity sizes are very similar. The Xavier Molecular Structure and Modeling Core was utilized to compare the ligand-binding cavity sizes of the 62 processed structures. At the same time, a manual sorting was undertaken to group ER LBD crystal structures by bound ligand type, cavity size and position of helix 12. This process resulted in the identification of 26 structures in the antagonist configuration (helix 12 open) and 36 structures in the agonist configuration with helix 12 closed. While cavity volume did not clearly group these structures, a combination of cavity size and bound ligand type was used to select representative agonist and antagonist crystal structures of the ER LBD. These 5 agonist structures (1ERE, 2G50, 2P15, 2QH6, 3ERD) and 3 antagonist structures (1ERR, 3DT3, 3ERT) will be used in the structure based database screening.

2c - Develop pharmacophore models from representative LBD structures (Months 1-4)

Since all of the ligands in the selected ER LBD structures bind to the ER using similar interactions, the development of classical pharmacophore models for the ER LBD models was determined to be unnecessary. Structure based screening for this project will utilize docking to the selected crystal structures where typical pharmacophore interactions are part of the ligand pose generation and score process.

Task 3- Mine phytochemical and marketed drug databases with pharmacophore models. (Months 3-5)

3a - Evaluate Docking methods for virtual screening of estrogens (months 4-8)

The Xavier Molecular Structure and Modeling core was utilized to quickly evaluate the potential for the MOE, Gold, Glide and Surflex Dock to be used for docking into the ER LBD. FlexX is nolonger used in this lab and the Glide method is and additional

methods used in the modeling core. This fast study using default setting of the software to replace the bound ligand into the binding cavity did not identify significant differences in the performance of these methods. Considering that the Wiese lab has the most experience with the MOE software and the fact that the MOE software is the only docking package available to us that can include a force field optimization, MOE was selected for further optimization studies.

3b - Ligand replacement optimization docking of representative LBD structures using MOE, Gold, FlexX, Surflex Dock (months 4-7)

A systematic ligand replacement study was performed using the MOE software and an optimal configuration of the MOE docking method was identified hat produced ligand replacement very close to the crystal structure (low RMSD).

The phytochemical (76,451 compounds) and marketed drug databases (16,096 compounds) were obtained from the Xavier Molecular Structure and Modeling core in SDF format. The process of processing these databases for virtual screening is currently underway. This process includes creating all tautomers, isomers, enantiomers and filtering out compounds too large to bind ER using MOE. In addition, the software Meteor will be used to create potential Phase I metabolites of each structure.

Task 4- Refine pharmacophore selection of estrogens using docking (months 6-8)

This process has not yet started.

Task 5- Hire research associate to assist with in vitro assays (Month 7)

Candace Hopgood has worked in the Wiese lab for 2 years and was transferred to this project briefly in spring and summer 2012. She spent the summer testing some of the bioassays to be used in the validation phase of this project including the Lantha Screen ER binding assay and the labs MVLN and T47D reporter gene assays. Candace left the lab August 31 and may be replaced in fall 2012 or early 2013 when the modeling phase of the project has selected enough compounds for validation. In the mean time, another Xavier Pharmacy Center of Excellence student, Felicia Gibson, has taken over bioassays for other projects in the Wiese lab and is ready to work on validation studies for this project.

Sridhar/Jones/Stevens Subproject (Identification of a New Class of Tyrosine Kinase Inhibitors)

The research accomplishments of this subproject include the following:

Task 1- Hire research associate to assist in project (Month 1)

Dr. Jayalakshmi was hired to work on this project. Her expertise in organic chemistry and skills in molecular modeling made her an ideal fit for this project. In

August 2012, she began a position as Assistant Professor of Chemistry at Xavier University. In her new capacity as Assistant Professor, she will co-direct this project with Dr. Cheryl Stevens who has left Xavier for a position as Dean of the College of Science and Engineering at Western Kentucky University. Dr. Stevens and Dr. Sridhar have agreed to continue collaborating on this project with the goal of developing Dr. Sridhar into a prolific and well-trained cancer researcher.

Task 2- Identify student to assist in project (Month 3)

Due to Dr. Stevens leaving Xavier University in January 2012, no students were hired on this project.

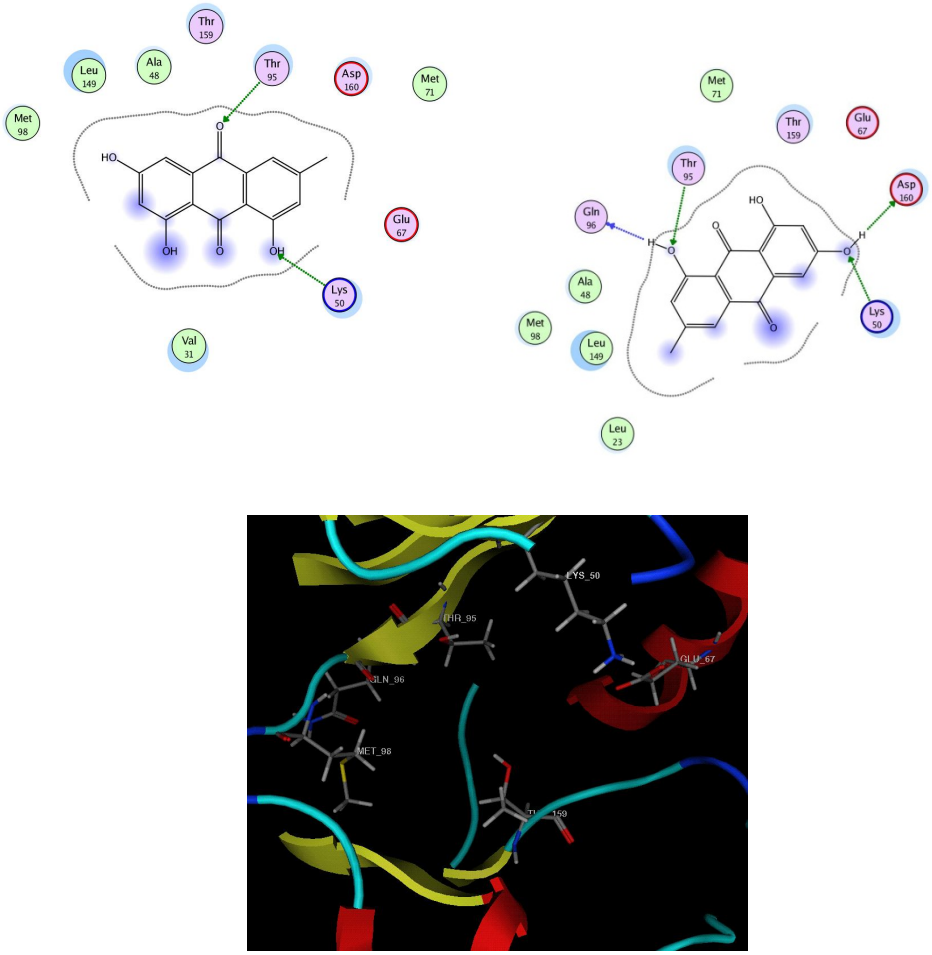
Task 3- Identify novel small molecules related to quinazoline, tyrphostin, emodin, and dasatinib that inhibit HER2 activity (Months 1-24)

3a - Identify detailed pharmacophore and determine geometric, electronic, and lipophilic characteristics required for tyrosine kinase inhibition (Months 1-12)

HER2 is a growth factor receptor protein belonging to the tyrosine kinase receptor family. HER2 is overexpressed in 25-30% of breast cancer patients and its overexpression has been detected in several other cancers including prostate cancer, ovarian cancer, lung cancer, mammary carcinoma, liver tumors and colorectal cancers. Trastuzumab is a humanized antibody targeting the extracellular domain of HER2 that is currently being used clinically. Among the many tyrosine kinase inhibitors developed so far, only Lapatinib is in clinical use. Several other HER2 kinase inhibitors are in various stages of clinical trials.

The splice variant HER2Δ16 isoform lacking exon 16 preceding the transmembrane domain shows low sensitivity to Trastuzumab. This makes the development of a HER2 kinase inhibitor a more reasonable approach. Castiglioni, et al., (*Endocrine-Related Cancer* (2006) 13, 221-232) have shown that emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) was the only drug that inhibited the therapeutically resistant oncogenic HER2 isoform, HER2Δ16. Based on these reports, emodin was taken as the lead structure for development of HER2Δ16 inhibitors. Emodin and Iressa were first docked onto the HER2 homology model to study their binding modes with the help of MOE docking tools. Iressa did not bind to the hinge region residues of the protein. Emodin did bind to the hinge region of the protein and three binding modes were identified (Figure 1). Based on the orientation of emodin in the binding pocket of the protein, residues that could be targeted for developing a good inhibitor were identified. These were Thr95, Gln96, Met98, Asp160, Lys50, Glu67, Thr159 (Figure 1).

Figure 1. Binding modes of emodin onto HER2 protein homology model and a picture of the binding pocket with the potential target residues depicted in stick mode.



3b - Identify new compounds to be tested for tyrosine kinase inhibition with conformationally flexible searches of compound databases using detailed pharmacophore and CoMFA QSAR results. (Months 9-24).

1,3,8-trihydroxyanthraquinone was taken as the pharmacophore for a UNITY 2D-search of all the databases available to us. Hits were obtained from ACD (10 hits), TSCA (3 hits) and NCI databases (39 hits). NCI database hits overlapped significantly with the compounds contained in ACD hits.

Task 4- Explore the mechanism of HER2 tyrosine kinase inhibition (Months 12-48)

4a - Dock proven and proposed TKIs into the tyrosine kinase ATP binding site using multiple poses, and score results (Months 12-24)

All of the hits described in **3b** were then docked onto the homology model of HER2 using MOE dock tools. The docking results were then studied manually. Binding of the molecule to one of the hinge region residues THR95, GLN96, MET98 was taken as a prerequisite. The number of ligand-protein hydrogen bond interactions, the extent of penetration of ligand into the pocket and the nature of ligand solvent exposure (hydrophobic/hydrophilic) were also considered.

4b - Optimize molecular structures to maximize ability of compounds to inhibit HER2 (Months 15-30)

No progress yet.

4c - Attempt to identify alternate binding sites (Months 18-30)

No progress yet.

4d - Perform *in vitro* kinase inhibition and binding assays (Months 18-48)

A total of 28 compounds were procured from the Developmental Therapeutics Program NCI/NIH and Specs chemicals. An initial high-throughput assay was performed to determine the inhibition of proliferation of MCF-7 cell line. The compounds that showed good inhibition activity were then subjected to in-vitro assay against HER2Δ16 cell line activity. Two compounds that showed low inhibition activity were included to confirm the activity profile of this set of compounds. Two of the tested compounds (NSC322354 and AG-650-41069319) showed low micro molar activity against the HER2Δ16 cell line (< 10 mM) (Table 1).

Table 1. Proliferation of MCF-7 cell lines- a high-through put assay

No.	**Compound**	**% of E2 at 10^{-5}M**	**IC_{50} HER2Δ16 (μM)**
1	AG-650/41069241	91.27	
2	AG-650/41069319	11.11	10.3
3	AG-650/41069355	98.16	
4	AP-782/41885488	0.26	34.4
5	AQ-776/42801622	87.79	40.59

6	AE-508/36399063	95.19	
7	AP-782/21243033	96.81	
8	AN-967/15488023	95.16	
9	AG-650/41069356	0.00	217
10	AE-848/13198350	100	
11	NSC322354	0.04	9.52
12	NSC227279	0.10	20.5
13	NSC109351	52.35	17.67
14	NSC202069	95.71	
15	NSC299384	74.26	
16	NSC309875	88.56	
17	NSC309876	89.11	
18	NSC310337	82.17	
19	NSC310338	94.35	
20	NSC319437	82.20	
21	NSC367088	88.97	
22	NSC379572	96.60	
23	NSC379866	96.79	
24	NSC93419	18.04	23.8
25	NSC7794	100.94	
26	NSC138557	80.50	50.8
27	NSC204855	9.11	22.7

28	NSC251670	101.55	

The compound NSC-322354 which showed the best inhibition activity against HER2delta16 cell line was taken for an analysis of its cross kinase activity. The compound was sent to KinomeScan (www.kinomescan.com) for KINOMEscan's in vitro competition binding assay against a panel of 96 representative kinases. KINOMEscan™ is based on a competition binding assay that quantitatively measures the ability of a compound to compete with an immobilized, active-site directed ligand. The assay is performed by combining three components: DNA-tagged kinase; immobilized ligand; and a test compound. The ability of the test compound to compete with the immobilized ligand is measured via quantitative PCR of the DNA tag (description of method taken from www.kinomescan.com). The compound was tested at a concentration of 10 mM. The results are given in Table 2. The S-score, selectivity score (the number of kinases that bind to the compound divided by the total number of distinct kinases tested), which is a quantitative measure of the compound selectivity, was 0.022. The compound showed good selectivity for two of the kinases, Casein Kinase 1 D and PIM kinases (more selective for PIM1 and PIM3 kinases). Both of these kinases are serine/threonine kinases. PIM1 is an oncogene. The PIM1 gene was initially identified as a proviral integration site in Moloney Murine leukemia virus-induced mouse T-cell lymphomas (Cuypers, H. T. *et. al*., *Cell* **1984,** *37*, 141; Dhanasekaran, S. M. *et. al.*, *Nature* **2001,** *412*, 822). Pim kinases are implicated in the development of solid tumors. DNA microarray analyses showed the overexpression of PIM1 in human prostate cancer in relation to the grade of the prostate cancer. CK1d is a member of the ubiquitous casein kinase-1 family, and alterations in the expression and/or activity of CK1 have been observed in breast cancer (Giamas, G. *et. al.*, *Nucleic Acids Res.* **2009**, *37*, 3110). CK1d, has been identified as a novel kinase implicated in the modulation of physiological aspects of both ERa (estrogen receptor alpha) and AIB1 (amplified in breast cancer-1 protein). The compound in fact did not show good inhibition of HER2 (ERBB2) kinase. The *in-vitro* high-throughput assay of the compounds is currently being performed for the proteins PIM1 kinase, and casein kinase 1 D. This will be followed by a dose response curve assay to determine the IC_{50} value for these kinases. These compounds will also be tested for inhibition of other breast cancer cell lines such as T47D, and MDAMB231.

Table 2. Matrix of Compound NSC322354 Screen

Kinase target	%Control @ 10μM		Kinase target	%Control @ 10μM
ABL1(E255K)-phosphorylated	96		KIT(V559D,T670I)	86
ABL1(T315I)-phosphorylated	95		LKB1	100
ABL1-phosphorylated	88		MAP3K4	100
ACVR1B	90		MAPKAPK2	93
ADCK3	100		MARK3	100
AKT1	100		MEK1	92
AKT2	100		MEK2	96
ALK	100		MET	100
AURKA	87		MKNK1	45
AURKB	72		MKNK2	98
AXL	100		MLK1	99
BMPR2	60		p38-alpha	100
BRAF	87		p38-beta	92
BRAF(V600E)	70		PAK1	93
BTK	100		PAK2	57
CDK11	86		PAK4	91
CDK2	92		PCTK1	100
CDK3	88		PDGFRA	100
CDK7	88		PDGFRB	100
CDK9	100		PDPK1	87
CHEK1	79		PIK3C2B	100
CSF1R	77		PIK3CA	99
CSNK1D	30		PIK3CG	68
CSNK1G2	100		PIM1	34
DCAMKL1	90		PIM2	67
DYRK1B	53		PIM3	36
EGFR	88		PKAC-alpha	84
EGFR(L858R)	78		PLK1	96
EPHA2	100		PLK3	54
ERBB2	93		PLK4	89
ERBB4	95		PRKCE	92
ERK1	100		RAF1	100
FAK	95		RET	100
FGFR2	100		RIOK2	86
FGFR3	93		ROCK2	77
FLT3	84		RSK2(Kin.Dom.1-N-terminal)	53
GSK3B	85		SNARK	90
IGF1R	100		SRC	91
IKK-alpha	87		SRPK3	93
IKK-beta	91		TGFBR1	100
INSR	97		TIE2	89
JAK2(JH1domain-catalytic)	94		TRKA	78
JAK3(JH1domain-catalytic)	61		TSSK1B	89
JNK1	96		TYK2(JH1domain-catalytic)	57
JNK2	84		ULK2	88
JNK3	76		VEGFR2	100
KIT	76		YANK3	89
KIT(D816V)	100		ZAP70	100

Task 5- Determine preclinical activity and specificity of novel HER2-targeting molecules: determine influence of targeting molecules on HER2 oncogenic signaling and cellular responses using multiple validated preclinical models of breast tumorigenesis and metastasis (Months 12-24)

5a - Perform genome wide shRNA library screen coupled with gene expression arrays of sensitive cells to identify Drug Targets, Drug Sensitizers, and Drug Resistance Pathways (Months 18-30)

No progress yet.

5b - Identify and validate drug combinations to improve efficacy and overcome resistance in preclinical models (Months 24-36)

No progress yet.

5c - Confirm efficacy of drug combinations in preclinical *in vivo* xenograft and transgenic mouse models of breast cancer (Months 30-48)

No progress yet.

Programmatic Activities

The breast cancer research group has met three times to discuss and plan the various aspects of the project. The PI and Co. PIs met in Fall 2011 to plan for project implementation. In July 2012 the whole group (all faculty, staff, and students involved in the project) met for a pizza lunch. On September 17, Dr. Matthew Burow presented a seminar/workshop to the group titled "Glyceollins – Development of Natural Product Based Anti-Estrogenic and Anti-Cancer Agents". Two drug discovery/translational research seminars have been scheduled for October 15 and 22, 2012. Subsequent meetings and workshops will be scheduled throughout the year with Xavier and Tulane alternating as venues. The researchers (faculty, staff, and students) of each subproject have met multiple times during the past year to discuss the progress of their specific research projects.

KEY RESEARCH ACCOMPLISHMENTS:

Foroozesh/Beckman/Burow Subproject

- Successfully developed a facile synthetic route to prepared 3-ketone-4,6-diene and 1-position modified ceramide analogs, and obtained 16 novel ceramide analogs.
- Discovered a highly potent ceramide analog (406). The mechanism investigation showed that analog 406 leads to cell apoptosis through intrinsic apoptotic pathway and does not interrupt the function of GCS.
- Designed, synthesized, and determined a novel GCS inhibitor (503, a 1-position modified ceramide analog), which is extremely useful for the development of highly potent GCS inhibitors.

Wiese/Burow Subproject

- Identified representative ER LBD structures to be used for virtual screening
- Identified optimal ligand receptor (Docking) method for virtual screening
- Obtained phytochemical and marketed drug databases for processing
- Trained two pharmacy students, one in molecular modeling, one in bioassays

Sridhar/Jones/Stevens Subproject

- Identified the residues in the HER2 ATP binding pocket that need to be targeted by the inhibitor.
- Identified a pharmacophore that could be used to search databases to give new compounds that potentially inhibit the therapeutically resistant oncogenic HER2 isoform, HER2Δ16.
- Identified a new compound with good selectivity for two kinases.
- Identified compounds that inhibit MCF7 breast cancer cell line.

Program Accomplishments

- Introduction of students, staff, and faculty members working on the different subprojects.
- Organization of training workshops/seminars.

REPORTABLE OUTCOMES:

- **Publications**

Foroozesh/Beckman/Burow Subproject

"Design, Synthesis, and Characterization of 4,6-Diene-ceramide Analogues for the Treatment of Chemo-resistant Breast Cancer" to be submitted to the *Journal of medicinal Chemistry* in September 2012.

Wiese/Burow Subproject

None yet.

Sridhar/Jones/Stevens Subproject

None yet.

- **Presentations**

Foroozesh/Beckman/Burow Subproject

"Novel Ceramide Analogs for the Treatment of Breast Cancer", A.P. Ponnapakam, B.A. Drew, T.L. Wang, L. Liu, M. Foroozesh, and B.S. Beckman, Advances in Breast Cancer

Research: Genetics, Biology, and Clinical Applications Conference, American Association for Cancer Research, San Francisco, CA, October 2011.

"Emodin Analogs as Cytochrome P450 Inhibitors- A Study of Their Potency and Selectivity", J. Sridhar, J. Liu, C.L.K. Stevens, and M. Foroozesh, Society of Toxicology National Meeting, San Francisco, CA, March 2012, and Louisiana Cancer Research Consortium Annual Retreat Meeting, March 2012.

"Novel Ceramide Analogs for the Treatment of Breast Cancer", A.P. Ponnapakam, B.A. Drew, T.L. Wang, J. Liu, M. Foroozesh, and B.S. Beckman, Louisiana Cancer Research Consortium Annual Retreat Meeting, March 2012.

Wiese/Burow Subproject

None yet.

Sridhar/Jones/Stevens Subproject

"Design and Development of New Pim1 Kinase Inhibitors", Jayalakshmi Sridhar, Ian Townley, Thomas Wiese, and Cheryl L. Klein Stevens, Louisiana Cancer Research Consortium Annual Retreat Meeting, March 2012.

- **Employment or Research Opportunities**

Individuals trained in the first year of this DoD breast cancer project:

Jiawang Liu, Postdoctoral Fellow at Xavier University (Foroozesh Lab)

Jayalakshmi Sridhar, Postdoctoral Fellow at Xavier University (Stevens Lab, currently a new tenure-track faculty member at the Xavier University Department of Chemistry)

James Antoon, Medical Student at Tulane University (Beckman Lab, received his M.D. in May 2012 after receiving his Ph.D. in 2010 at Tulane University. He is currently doing his residency in pediatrics at the University of North Carolina.)

Barbara Drew, Medical Student at Tulane University (Beckman Lab, is currently in her residency in obstetrics and gynecology in Connecticut.)

Tony Wang, Medical Student at Tulane University (Beckman Lab, working on this DoD subproject)

Thong T. Nguyen, Undergraduate Student at Xavier University (Foroozesh Lab, graduated in May 2012 and is now pursuing a Ph.D. in Chemistry at Tulane University)

Adharsh P. Ponnapakam, Undergraduate Student at Tulane University (Beckman Lab, graduated in May 2012 and is currently continuing his work on this DoD project as a Masters student at Tulane University)

Patrick Dupart, Technician at Xavier University (Foroozesh Lab, Xavier graduate)

Shannon Taylor, Technician at Xavier University (Foroozesh Lab, Xavier graduate)

Corey Arnold, Undergraduate Student at Xavier University (Foroozesh Lab)

Candace Hopgood, Technician at Xavier University (Wiese Lab, Xavier graduate)

Chioma Obih, Pharmacy Student at Xavier University (Wiese Lab)

Felicia Gibson, Pharmacy Student at Xavier University (Wiese Lab)

Elizabeth Martin, Graduate Student at Tulane University (Burow Lab)

Felicia Huynh, Graduate Student at Tulane University (Jones Lab)

Hope Burks, Graduate Student at Tulane University (Burow Lab)

Lucas Chan, Masters Student at Tulane University (Beckman Lab)

Lyndsay Rhodes, Postdoctoral Fellow at Tulane University (Burow Lab)

Melyssa Bratton, Instructor at Tulane University (Burow Lab)

Steven Elliott, Lab Supervisor at Tulane University (Burow Lab)

Van Hoang, Graduate Student at Tulane University (Burow Lab)

Han Wen, Graduate Student at Tulane University (Jones Lab)

CONCLUSION:

Foroozesh/Beckman/Burow Subproject

Our results have shown that extending the conjugated system in the backbone of ceramide analogs can lead to an increase in the anti-cancer activity. This conclusion will assist us in designing more potent anti-cancer ceramide analogs.

We have also found that the modification of the 1-position of ceramide can lead to novel glucosylceramide synthase (GCS) inhibitors. This finding provides us with a new perspective for the design of effective GCS inhibitors.

Wiese/Burow Subproject

In year one, we have developed the methods we will use to perform virtual screening of the phytochemical and marketed drug databases. This involved obtaining all crystal structures of the ERalpha ligand binding domain, sorting these structures by ligand type and structure characteristics, and then comparing and optimizing ligand

receptor docking protocols. At the same time, we obtained the phytochemical and marketed drug databases and started the process of filtering for compounds with potential to bind ER that will go into the virtual screening process. Two pharmacy students were trained and then involved in the molecular modeling as well as trained for the in vitro validation phase of the project.

Sridhar/Jones/Stevens Subproject

Over the past year we have been able to identify molecules that target two kinases, namely, PIM1 and CK1d, which play important roles in prostate cancer and breast cancer. Several compounds were found that inhibited MCF7 breast cancer cell line and HER2Δ16. Development of these lead compounds using molecular modeling and organic synthesis will give us potential drug candidates for breast cancer and prostate cancer. The dose response curve studies are ongoing for these two kinases. Based on the results further modification of the lead molecules will be attempted towards the goal of achieving better potency and selectivity for these two kinases. In the meantime, new database searches will be initiated based on the docking studies of known kinase inhibitors on HER2 to identify new core structures as lead molecules with the final goal of finding a new drug candidate for breast cancer.

Program

Other than the significant amount of scientific research performed and data collected during this first year of the project, it is important to note the valuable partnership developed between the two institutions involved. The productive collaboration formed between the Xavier University and Tulane Cancer Center researchers participating in this program, once again proves the value and importance of inter-institutional research/training projects. The different training activities and the number of trainees involved in the various aspects of the subprojects also positively impact the future cancer research environment in the area. This breast cancer research project is still in its early stages and is expected to develop significantly over the next years.

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Wiese/Burow Subproject

NA

Sridhar/Jones/Stevens Subproject

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